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RESEARCH PAPER

Acacia senegal Possesses *in vitro* Cytotoxicity Against Human Cancer Cells

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ABSTRACT : *In vitro* assay for cytotoxic activity of *Acacia senegal* has been carried out against four human cancer cell lines from four different tissues *via* aqueous extract at the concentration of 10, 30 and 100 µg /ml using Sulphorhodamine blue (SRB) assay. Results revealed that fruit and seed part of the plant showed remarkable *in vitro* cytotoxic potential against three human cancer cells of cervical (SiHa), neuroblastoma (IMR-32) and lung (A-549) origin at the concentration of 30 and 100 µg/ml. Based on *in vitro* data, it is suggested that further *in vivo* studies as well as identification of effective components could be useful in designing new anti-cancer therapeutic agents from this particular plant.

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Acacia senegal, Human cancer cells, Neuroblastoma

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Acacia senegal, known as gum arabic and belonging to family Leguminosae, is distributed mainly in tropical and sub tropical regions of southern part of West Pakistan and India (specifically in Jaipur and Jodhpur), the species grows to 2-15m tall with a flat or rounded crown. The plant possesses phytoconstituents like flavone, catechin, polyphenols, tannins, chalcones, alkaloids and flavonoids (Majekodunmi *et al*., 2006). Gum arabic exudates offers protection against cyclophosphamide induced urinary bladder cytotoxicity (Adel *et al.,* 2009). Recent studies have highlighted its antioxidant properties (Trommer and Neubert, 2005; Ali and Al Moundhri, 2006 and Hinson *et al*., 2004) and its positive results used in treatment for several degenerative diseases such as kidney failure (Matsumoto *et al.,* 2006 and Ali *et al.,* 2008), cardiovascular (Glover *et al.,* 2009) and gastrointestinal (Wapnir *et al.,* 2008). In the present study, the fruit and seed part of the plant has been evaluated against four human cancer cells from four different tissues.

RESEARCH METHODOLOGY

Chemicals :

RPMI-1640 medium, dimethyl sulfoxide (DMSO), EDTA, fetal bovine serum (FBS), sulphorhodamine blue (SRB) dye, phosphate buffer saline (PBS), trypsin, gentamycin, penicillin and 5-flurouracil were purchased from Sigma Chemical Co., USA. All other chemicals were of high purity and obtained locally with the brand Sigma-Aldrich Chemicals Pvt. Ltd. and S.D. Fine

Chemicals Pvt. Ltd. from Ramesh Traders, Panjthirthi-Jammu, J&K.

Preparation of extract :

A lot of dried plant material (100 g) was heated with 6 to 8 times its weight of distilled water on a steam bath in a conical flask for 4 to 5 h. The extract was decanted/ drained and filtered depending on the need. The process was repeated 4 times for complete extraction. The combined extract was freeze dried. The extract was then transferred to a tared wide mouth glass container. The plant extract obtained, was stored at -20^0 C under desiccation in deep freezer for further testing. Stock solutions of 20 mg/ml were prepared by dissolving extract in distilled water. Stock solutions were prepared at least one day in advance and were not filtered. The microbial contamination was controlled by addition of 1 per cent gentamycin in complete growth medium *i.e.* used for dilution of stock solutions to make working test solutions of 200 μg/ml.

Cell lines / cultures and positive controls :

The human cancer cells were obtained from National Centre for Cell Science, Pune, India and National Cancer Institute, Frederick, USA. These human cancer cells were further grown and maintained in RPMI-1640 medium. Adramycin, 5-Fluorouracil and Paclitaxel were used as positive controls.

***In vitro* assay for cytotoxic activity :**

Extracts were subjected to *in vitro* anticancer activity against various human cancer cell lines (Monks *et al.*, 1991). In brief, the cells were grown in tissue culture flasks in growth medium at 37°C in an atmosphere of 5 per cent CO_2 and 90 per cent relative humidity in a CO_2 incubator (Hera Cell, Heraeus; Asheville, NCI, USA). The cells at sub-confluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97 per cent viability (trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 μl of cells (10^5 cells/ml) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Extracts (100 μl/well) were then added to the wells and cells were further allowed to grow for another 48 h.

The anti-proliferative SRB assay which estimates cell number indirectly by staining total cellular protein with the dye SRB was performed to assess growth inhibition. The SRB staining method is simpler, faster and provides better linearity with cell number. It is less sensitive to environmental fluctuations and does not require a time sensitive measurement of initial reaction velocity (Skehan *et al.*, 1990). In brief, the cell growth was stopped by gently layering 50 μl of 50 per cent (ice cold) trichloroacetic acid on the top of growth medium in all the wells. The plates were incubated at 4°C for 1 h to fix the cells attached to the bottom of the wells. Liquid of all the wells was then gently pipetted out and discarded. The plates were washed five-times with distilled water and air-dried. SRB 100 μl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the cells five-times with 1 per cent acetic acid. Plates were air-dried, tris buffer (100 μl, 0.01 M, pH 10.5) was added to all the wells to solubilize the dye and then plates were gently stirred for 5 min on a mechanical stirrer. The optical density (OD) was recorded on ELSIA reader at 540 nm. Suitable blanks (growth medium and DMSO) and positive controls (prepared in DMSO and distilled water) were also included. Each test was done in triplicate and the values reported were mean values of three experiments.

The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Per cent growth in presence of test material was calculated as under:

- OD change in presence of control = Mean OD of control – Mean OD of blank
- OD Change in presence of test sample = Mean OD of test sample – Mean OD of blank
- Per cent growth in presence of control = 100/ OD change in presence of control
- Per cent growth in presence of test sample = per cent growth in presence of control × OD change in presence of test sample
- Per cent inhibition by test sample = 100 – per cent growth in presence of test sample

The growth inhibition of 70 per cent or above was considered active while testing extracts.

RESULTS AND DISCUSSION

The aqueous fruit and seed extract from *A. senegal* (100 μg/ml) showed *in vitro* anticancer potential against

Table 1 : Growth inhibitory effect of fruit and seed part of *Acacia senegal* against human cancer cell lines along with positive controls

Generic name of the plant	Extract	Conc. (µg/ml)	Human cancer cell lines from four different tissues			
			A-549	SiHa	502713	IMR-32
			Lung	Cervix	Colon	Nueroblastoma
Acacia senegal	Aqueous		Per cent growth inhibition			
		10	44	55	30	27
		30	71	88	66	83
		100	99	92	95	99
Positive control		Conc. (M)				
5-Fluorouracil		$1x10^{-5}$ M	55	13	41	42
Paclitaxel		$1x10^{-6}$ M	63	31	87	59
Adriamycin		$1x10^{-6}$ M	89	47	85	98

Growth inhibition of 70 per cent or more in case of extracts has been indicated in bold numbers
Mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

four human cancer cell lines as the growth inhibition was observed in the range of 92-99 per cent. The extract showed 99 per cent growth inhibition of lung (A-549) and neuroblastoma (IMR-32) human cancer cells. Moreover, 95 per cent and 92 per cent growth inhibition was observed against colon (502713) and cervical (SiHa) cancer cell lines. When evaluated at lower concentrations (30 µg/ml), the same aqueous extract inhibited the proliferation of three human cancer cell lines from cervical, neuroblatoma and lung origin. The extract showed 88 per cent growth inhibition of cervical, 83 per cent of neuroblastoma and 71 per cent of lung cancer cells. However, at 10 µg/ml the extract did not exhibit any significant activity (Table 1). Cancer is becoming a big load on families and economies. Therefore, the research for alternative drugs of natural origin, which are less toxic, endowed with fewer side effects and more potent in their mechanism of action, is an important research line. Plants have long history for the treatment of various diseases including cancer and active principles from these plants are used to control the advance stages of malignancies in clinical settings. These natural products now have been contemplated of exceptional value in the development of effective anticancer drugs with minimum host cell toxicity. A number of exciting researches suggest that vegetables, fruits, whole grains, herbs, nuts and seeds contain an abundance of polyphenolic compounds, terpenoids, sulphur compounds, pigments and other natural antioxidants, that have been associated with protection from or treatment of conditions such as cancer.

Therefore, we can say that natural products have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. The results obtained from our investigation confirmed the therapeutic potency of *A. senegal* especially against cervical cancer cells. The results also showed that this plant possesses certain cytotoxic constituents that can be used for developing anticancer agents for cervical cancer therapy. Moreover, it forms a good basis for the selection of fruit and seed part of the plant for further phytochemical and pharmacological analysis and offer us new drugs from natural sources which would be less toxic and more potent for the efficient management of cancer. To conclude active ingredients from the aqueous extract of *A. senegal* can act as lead molecules to provide a great service and promise to cancer patients.

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